Guest Editorial

Detection of Novel Viruses

Rachael E. Tarlinton, *Stephen P Dunham

School of Veterinary Medicine and Science
University of Nottingham,
Sutton Bonington Campus
Loughborough, LE12 5RD, UK

*Corresponding author. Tel: +44 (0)115 9516580 fax: +44 (0)115 9516440
Email address: Stephen.dunham@nottingham.ac.uk

Next generation sequencing methods have the potential to transform the study of normal biological processes and disease in humans, animals and microbial populations (Kahvejian et al., 2008). This extends to the identification of novel pathogens, and given the recent flurry of research activity in this area, the review of detection methods for novel viral nucleic acid by Bexfield and Kellam in this issue of the Veterinary Journal is extremely timely. The article provides an excellent summary of the main molecular techniques used in viral sequence discovery and highlights associated success stories. The article concludes with a reminder that the identification of a virus in an individual with disease is not the same as proving an association with pathogenesis.

This is a particularly vexing problem for groups of viruses such as herpesviruses and retroviruses that can be regarded as ubiquitous in vertebrates. The degenerate PCR protocol developed by (VanDevanter et al., 1996) has proved to be extremely robust in identifying herpesviral sequence in a range of different vertebrates. These include bats, rodents, primates, elephants, dolphins, reptiles and birds (Ehlers et al., 2001; Ehlers et al., 2007; de Thoisy et al., 2009; Wibbelt et al., 2007). Indeed our own experience with these primers would suggest that it is only rarely that herpesvirus sequences cannot be isolated from a vertebrate species (Tate, 2009). The biology of these viruses is complex and there has been some suggestion that they form in essence a "viral normal flora" in their hosts (Brahic, 2010). Some of these papers have reported the finding of herpesviral sequence in diseased animals, the strongest association being between elephant endotheliotrophic herpesvirus and fatal haemorrohagic disease in young elephants (Garner et al., 2009). However with an unculturable virus demonstration of disease causality relies on a statistical association between the putative pathogen and the disease syndrome rather than the fulfilment of classical Koch's postulates.

The situation with retroviruses is even more confusing. These viruses insert copies of themselves into their hosts DNA with these "endogenous" viruses becoming an inherited part of their host's genetic makeup. In many cases these become degenerate and incapable of functioning as a virus but in many species including mice, cats and chickens they are similar enough to their infectious "exogenous" counterparts that they may swap envelope genes (Roy-Burman, 1995). Without very carefully designed nucleic acid detection methods it can be very difficult to distinguish endogenous from exogenous viruses (Patience et al., 2001). This is of particular interest in the ongoing controversy surrounding the identification of xenotropic murine leukaemia related virus (XMRV) sequence in various human pathologies including prostate cancer and chronic fatigue syndrome (Weiss, 2010). The fact that the findings of the original papers (Lombardi et al., 2009; Urisman et al., 2006) have not been able to be reproduced by others and the lack of biological "sense" in the proposed disease association (exogenous viruses are commonly associated with immunosuppression and lymphomas not reproductive tract cancers and neurological syndromes) strongly suggests contamination with DNA containing an undescribed retrovirus in these studies.

The fact that scenarios similar to the XMRV story have been reported before (Griffiths et al., 2002) highlights the need for caution when implying disease causation by a novel virus. The advent of next generation sequencing technologies will increase the likelihood of such reports, as the sheer volume of sequence data generated and sensitivity of the technique makes it more likely that contaminants or non-significant virus sequences will be amplified. Interestingly despite many years of study on endogenous retroviruses and the availability of complete genome sequences for many of the common laboratory, agricultural and companion animals there has been little interest in characterising the retroviral complement (whether endogenous or exogenous) in these animals. Groups have focussed on particularly active human clades (like HERV K) (Moyes et al., 2007) or the JSRV like group

in sheep (Black et al., 2010) which have a known exogenous counterpart. However recent reports of murine retroviral diversity (Baliji et al., 2010; Kozak, 2010) in the wake of the XMRV papers highlights the lack of knowledge of these viruses, even in well studied species like the mouse. This is not helped by the chaotic naming schemes for retroviruses. When combined with the lack of annotation of endogenous retroviruses in the available genome data and the probable existence of exogenous counterparts of these viruses (Blomberg et al., 2009) it is highly probable that sorting real from contaminating viral sequence will be a significant challenge.

Our own experience with characterising the endogenous retroviruses of the dog and horse genome has highlighted such difficulties. Different search algorithms return different numbers and locations of loci for even quite closely related groups of viruses. The situation with the dog genome is especially of interest as there have been multiple past searches for a "dog lymphoma virus" – at least one of which was probably a murine leukaemia virus contaminant. Our work has identified a group of gammaretroviruses that are clearly specific to the dog genome and are more likely to be related to any putative dog exogenous virus (Barfoot, 2009). This is especially pertinent in the light of the fact that species jumps of retroviruses do occur but are a relatively rare phenomenon and it is far more likely that the exogenous viruses of a species will have evolved along with that species (Martin et al., 1999).

The advent of increasingly sensitive technologies for the detection of viral sequences reported in Bexfield and Kellams' review has been a boon to those interested in the taxonomy of virus evolution. While such techniques are a welcome addition to the armoury of those interested in the role of viruses in disease pathology, the considerable challenge for those attempting to discover novel viral pathogens remains to demonstrate causality with disease. While we have outlined such problems in the context of retroviruses and herpesviruses, they similarly apply to other families of virus.

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